VOLUME 3 NUMBER 7

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NUTRITION REVIEWS

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

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AUG 9 1945
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ERRATA

April 1945 issue: page 100, 2nd column, line 19 change reference to read: *Nutrition Reviews* **3,** *44 (1945)*)

page 102, paragraph 4, line 9 delete sentence: ***When 1.0 per cent of dl-phenylalanine was present, normal growth resulted.***

AUG 9 1945

NUTRITION REVIEWS

VOL. 3 JULY, 1945 No. 7

PATHOLOGY IN RELATION TO NUTRITIONAL RESEARCH

Pathology is that branch of biology which is concerned with all responses of living organisms to injurious conditions, inclusive of defective nutrition. Methods of investigation include chemical, physiologic, and morphologic procedures. Adequate mastery of these disciplines by one person is not possible and therefore the closest possible cooperation should be established between the morphologically trained pathologist and those engaged in nutritional research in which physiologic and chemical methods predominate. A general pathologist who studies life chiefly from morphologic aspects may well be appalled by the wasted opportunities represented by animals consigned to incinerators at the completion of carefully conducted experiments in nutritional fields.

Of particular interest to the morphologist are studies of the consequences of deficiencies of essential dietary factors whether these be mineral elements, vitamins, or other organic compounds because he reasons that in each instance a particular biochemical process is affected and that its suppression may result in visible structural changes.

At the very least, he can obtain for himself new premises for the evaluation of the significance of lesions encountered in routine histologic studies of postmortem material, particularly those from individuals who died in the period of active growth while, for the biochemist, he may be able to indicate, through the study of experimental animal material, the organ or tissue in which the *in vivo* chemistry has ceased to operate because of the deficiency.

Experimental deficiency studies have already brought to light notable species differences in metabolic processes. The independence of most species of dietary sources of vitamin C is explained by their ability to synthesize this substance. In the cases of vitamin E and of several components of the vitamin B-complex-pyridoxine, nicotinic acid, and pantothenic acid—there are considerable variations in the deficiency consequences in different species, some of which suggest differences in physiology of certain organs or tissues (Wolbach and Bessey, *Physiol. Rev.* **22,** *233 (1942)*). Knowledge of such species differences can probably be greatly extended through morphologic studies, with benefit to some interests of physiologists, biochemists, and physicians.

Without correlated morphologic studies, the results of deficiency experimentation are always open to misinterpretation. Vitamin A can no longer be regarded as an anti-infective vitamin since the "abscesses" described by nutritionists have proved to be glands or ducts distended with keratinized epithelial cells, the result of reparative replacement of atrophic epithelium by stratified keratinizing epithelium. The explanation of the hemorrhages of scurvy is no longer sought for in the blood since collagen formation is known to be at fault as an outstanding result of vitamin C deficiency. Failure of collagen formation in many locations, notably periosteum from bone and epiphyses from diaphyses in infants, results in weakness of structures and separation of tissues with bleeding in consequence of mechanical factors. The solution of the role of vitamin D in affecting blood calcium levels would be expedited if biochemists would give less attention to absorption of calcium from the intestinal tract and more consideration to the blood plasma in the light of the consequences of excessive administration of vitamin D upon bone and cartilage. Hypervitaminosis D produces great increase of blood calcium and, in the absence of adequate alimentary calcium,

resorption of bone takes place in maintenance of the blood calcium levels imposed by the hypervitaminosis. Growth sequences—both of endochondral and appositional bone formation—continue, but bone matrix deposited may not calcify in spite of the high blood calcium. Omission of vitamin D results in rapid lowering of the blood calcium with deposition of calcium salts in many soft tissues.

Excessive vitamin A administration to young rats and guinea pigs results in fracture of bones. The current opinion generally held is that the fractures are the result of a "toxic effect" causing fragility of bone. The explanation (unpublished) recently worked out in this laboratory is that there is an extraordinary acceleration of remodeling sequences in conformity to normal growth patterns. The remodeling takes place in spite of a retardation of linear growth of bones and is correlated with accelerated epiphyseal cartilage sequences. Those sequences retarded and suppressed in vitamin A deficiency are grotesquely and dramatically accelerated by the excess.

Bone not destined to undergo remodeling is not affected by hypervitaminosis A. In those regions where the normal patterns of growth require marked reduction in diameter of long bones as, for example, the upper end of the tibia, extensive to complete resorption of the cortical bone takes place. Concurrently, there is bone formation on the endosteal side. This new bone, while in volume commensurate with the normal pattern requirement, fractures because it does not, in the brief period of the experiment, acquire adequate firmness because of incomplete calcification and probably also because of incomplete condensation of the collagenous matrix. Herein lies a new tool for the anatomist who wishes to study growth patterns of bones of different species because it is possible to get the equivalent of a year's growth in a ten to fifteen day period.

Paralysis in vitamin A deficient animals until recently was regarded by a number of experimenters as the result of a direct degenerative effect upon nerve tissue. The explanation is that epiphyseal bone growth is retarded very promptly and before growth of soft tissues, including the central nervous system, is affected (Wolbach and Bessey, *Arch. Path.* **32,** *689 (1941)*). The disproportionate growth results in a central nervous system too large for its bony encasement and hence lesions of brain, spinal cord, and nerve roots through compression. Incidentally, this effect provides, for the anatomist, the easiest method of discovering the distribution of arachnoidal villi in animals, inasmuch as these microscopic structures become grossly visible because they become the sites of herniations of brain substance into veins and sinuses.

Elsewhere (*Science* **86,** *569 (1937); Physiol. Rev.* **22,** *233 (1942)*) consideration has been given to the possibilities afforded by the employment of vitamin deficiencies and their recovery phenomena in various biologic fields.

There is still much to learn and a few disputes to settle, regarding the structural consequences of straight inanition produced by inadequate amounts of a perfect diet.

As yet no one has been able to correlate the distribution and character of cutaneous lesions of any deficiency states with any known function of the skin or with normal morphologic sequences of the epidermis and its appendages. Success in this direction may well be an initial step toward acquiring important knowledge of the physiology of the skin. The distribution of the skin lesions resulting from deficiency states—for example, pyridoxine deficiency—indicates that variations in metabolism if not in physiology must exist in relation to different regions of the body surface.

At present the possibility still exists that the very labile and highly important substances of nerve sheaths—myelin—may be directly affected by deficiency of an accessory food factor. Should this prove to be

the case a direct approach to problems of myelin formation and maintenance might become possible which, of course, would be of great importance in neurophysiology and neuropathology.

A pathologist cannot avoid the conviction that vitamin E is concerned with metabolic processes involved in voluntary muscle kinetics, because the degeneration of voluntary muscle resulting from vitamin E deficiency does not occur in muscle put completely at rest, either by denervation or by cutting tendons (Pappenheimer, *Am. J. Path.* **15,** *179 (1939)*).

Complete microscopic studies of animals in many of the deficiencies have yet to be made. Studies of every tissue in complete and partial deficiency states, with adequate technics applied with particular reference to minor cytologic changes, are required if physiologists and biochemists are to be provided with the premises they need in their search for the biochemical systems in which essential substances are operative.

The study of nutrition has tremendous practical importance. Its methods have been largely those of applied chemistry, applied physiology, and—less commonly—applied pathology. A common goal of morphologists and biochemists is the best possible understanding of the organization of living matter. Cytologists as yet, in general, have not responded to the opportunities afforded by deficiency experimentation but even the crude cytologic observations made by pathologists give many indications for the direction of biochemists seeking to discover chemical systems in which vitamins and "trace" elements are operative. Deficiency experimentation may be regarded as a method of chemical microdissection of cells. Morphologic correlation, whenever possible, should be made because for life, structure and chemistry are inseparable.

In the interests of economy and advancement of knowledge, the biochemically trained nutritionist and the pathologist should join forces whenever possible, or the pathologist should have the privilege of selection from animals discarded by the nutritionist.

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GLOSSITIS AND CHEILOSIS IN DEFICIENCIES OF THE VITAMIN B-COMPLEX

The complexity of clinical manifestations of deficiency of the vitamin B-complex is scarcely imagined by physicians whose medical discipline has emphasized the usually more stereotyped pattern of the common acute infections and the degenerative diseases. A deficiency of many different essential components of food may give rise to a train of symptoms and signs, the specificity of which is a moot question. As a consequence of depletion, tissues may change in various ways depending on their inherent reactivity (hereditary influence), the degree of depletion, the rate of its occurrence, the singleness or multiplicity of substances lacking. All these are superimposed upon the never static metabolic processes of growth and senescence. Glossitis has long been known as a frequent clinical finding in deficiency of the vitamin B-complex while cheilosis was first stressed by Stannus (*Tr. Roy. Soc. Trop. Med. Hyg.* **5,** *112 (1912)*) as angular or marginal stomatitis associated with pellagra, and more recently was observed to appear in riboflavin deficient women and to respond to therapy with riboflavin after nicotinic acid had been given (Sebrell and Butler, *Pub. Health Reports* **53,** *2282 (1938)*). The perplexity aroused by the response of cheilosis to pyridoxine, to brewers' yeast, and to ascorbic acid after failure to respond to riboflavin was reported by Machella (*Am. J. Med. Sci.* **203,** *114 (1942)*) and the whole topic of the so-called ariboflavinosis syn-

drome has been discussed (*Nutrition Reviews* **1,** *327 (1943)*). To those who are unfamiliar with the arguments for classifying cheilosis as a fungus or bacterial infection, reference should be made to Finnerud's etiologic and clinical study of 100 cases of perlèche (*Arch. Dermat. Syph.* **20,** *454 (1929)*) which recounts interesting studies on the culture of Monilia from the lesions, the production of lesions following inoculation with the organisms, and the rapid response of spontaneously occurring lesions to local application of silver nitrate. While many points in Finnerud's study suggest a possible relationship to nutrition the complete dismissal of infectious agents in the cause of cheilosis, even as secondary factors, by most contemporary investigators leaves open a possible loophole which might help clarify some of the clinical vagaries of the condition.

Recent investigations from the Duke Clinic reported by Cayer, Ruffin, and Perlzweig (*South. Med. J.* **38,** *111 (1945)*) have centered around the relationship of the clinical signs—glossitis and cheilosis—to the biochemical state of the subject in terms of excretion and load test of many compounds of the B-complex. These investigators have followed the plan of hospitalizing all patients and giving a "diet low in vitamins but adequate in protein and calories." Laboratory tests were carried out for vitamin A, ascorbic acid, thiamine, riboflavin, nicotinic acid, "F_2," and pyridoxine using methods reported by Ruffin, Cayer, and Perlzweig (*Gastroenterology* **3,** *340 (1944)*). There were two control groups —one of 28 ward patients without cheilosis or glossitis, suffering chiefly from "neurasthenia." Thirty medical students served as ambulatory controls. There were thus three groups: (1) those with clinical evidence of deficiency of the vitamin B-complex (glossitis, with or without cheilosis); (2) a sick but not obviously deficient group; and (3) healthy young men.

None of the patients in this series had clinical indication of vitamin A deficiency and there was no evidence of severe depletion in the tests on blood plasma though the mean values for all patients were lower than for the student controls. No clinical evidence of scurvy existed and the mean plasma ascorbic acid levels were well within safe limits even though 5 patients and 4 students had no measurable plasma ascorbic acid. "The most striking differences were found in the nicotinic acid excretion." Patients with glossitis or cheilosis excreted less than the control patients, and much less than the students following the load test, and the means for those with cheilosis or glossitis fell below the suggested lower limit of normal. Riboflavin levels of both load and control urinary excretion were lower in the patients than in the students and there were no meaningful differences between those with or without glossitis and cheilosis. There was no correlation between control period excretion of thiamine or of pyridoxine and the occurrence of glossitis or cheilosis.

A glance at the clearly presented charts which give distribution and ranges as well as means of the pertinent biochemical data for the several groups compared, demonstrates the differences in mean values but wide scattering of points. Thus in none of the figures is all points from the normal (student) data above the line suggested as the lower limit of normal, and there is an even greater scattering above the normal line of those with obvious deficiency diseases. In other words none of the levels during control periods or following load doses of vitamins serves as a method of absolute indication of deficiency, in the sense that the presence of dextrose in the urine is diagnostic of diabetes mellitus until proved otherwise. Data presented in this study do not support the authors' statement that "laboratory determination of the various vitamin levels assist materially in evaluating the nutritional status of any given individual." Just where most help is needed, in the borderline or equivocal

case, laboratory tests are apt to be most ambiguous.

As a tool to classify groups, the tests show striking differences where the means are considered. These differences are greatest between the students and the patients, regardless of the clinical findings except that excretion of administered nicotinic acid amide was lower in patients with cheilosis or glossitis than in those without.

The finding of similar riboflavin levels in patients with and without cheilosis is emphasized by those workers as strong indication that deficiency of riboflavin was not responsible for the cheilosis. They stress the multiplicity of deficiencies causing cheilosis and glossitis—the fact that cheilosis usually responded following therapy with nicotinic acid amide, often failed to respond after therapy with riboflavin, but sometimes responded only to the whole B-complex in the form of yeast or liver extract.

It is difficult to resolve the conflicting testimony which comes from clinics in Durham, Augusta, Birmingham, Washington, Philadelphia, New Orleans, and other places regarding the specificity of various clinical signs associated with B-complex deficiency. A number of possible causes might be entertained: (1) The population groups in these several areas may be so different as to respond differently to identical depletions; (2) local environmental conditions, temperature, light, radiant energy, and other meteorologic conditions may influence the reactivity of the exposed tissues; (3) foods eaten in various sections may differ significantly in their content and proportions of many important vitamins, minerals, and other constituents; (4) the whole ensemble of signs and symptoms resulting from deficiencies of the B-complex vitamins may be produced through interference with apparently unrelated functions and mechanisms. Thus one might hypothesize that a specific deficiency of substance X damaged the alimentary canal so that it could less readily absorb substance Y; (5) a single hepatic function might be perverted by deficiency of any of several vitamin B-complex factors and thus invariably give rise to a definite clinical picture regardless of which factor was chiefly responsible; (6) alimentary functions appear to be influenced by several vitamin B-complex factors. Thus the biosynthesis of a factor X, deficiency of which is actually responsible for a sign, might be effected by administration of another factor Y, and a resulting therapeutic benefit attributed to the direct action of factor Y.

The foregoing discussion simply points out the complexity of the clinical problems encountered in nutrition. Until it is possible to make long term studies of patients in their natural habitat, analyzing aliquots of diets rather than taking dietary histories, controlling all possible factors which influence general health, metabolism, and alimentary function, it is likely that confusion will prevail. Nevertheless, the science of human nutrition has made spectacular advances in the last decade. The physician in the clinic is still a long way from the precision of the biochemist or the controls of the animal experimenter. For this reason studies such as Cayer, Ruffin, and Perlzweig have reported from the Duke Hospital, though they do not attempt to give a final answer, constitute a definite advance, and by continuing to question some of the assertions and interpretations of other investigators, emphasize the need for careful objective investigation in this perplexing field.

THE ROLE OF THE LIVER IN PRODUCING HEMOGLOBIN AND PLASMA PROTEIN

In an earlier review (*Nutrition Reviews* **3**, *137* (*1945*)) the concept of Whipple and associates of a "dynamic equilibrium" of the proteins was discussed. This visual-

izes a ready flow of plasma proteins out of the circulation into the tissues and the reverse. It is their belief that the liver is concerned with the production of many of the plasma proteins and with the maintenance of the normal levels of these proteins in the circulating blood. The liver in other words is "the master organ" for various protein metabolic activities. It stores protein, it makes protein (fibrinogen, prothrombin, and other globulins and albumins), and it aggregates amino acids and other nitrogenous materials coming from the gastrointestinal tract into protein.

A further communication from Whipple's laboratory is concerned with the role of the liver in producing hemoglobin and plasma proteins on diets rich in liver and iron (Whipple, Robscheit-Robbins, and Hawkins, *J. Exp. Med.* **81,** *171 (1945)*). Studies which seek an answer to this problem were made on dogs in which an Eck fistula was produced. The Eck fistula carries blood from the abdominal viscera directly to the inferior vena cava rather than via the portal vein through the liver. Thus the blood supply to the liver is limited to its arterial source and is reduced, according to Whipple, to but 25 to 35 per cent of normal. The Eck fistula liver, according to these investigators, is somewhat smaller than normal but there is no significant fibrosis. The liver cells here and there show fat droplets but for the most part appear normal. Such a liver may produce less bile salt than the normal control. Dogs with Eck fistulas have been maintained in an apparent state of health for as many as eight years although at times such dogs present unusual disturbances of protein metabolism. Much of the time they appear normal in all respects, activity, appetite, digestion, and weight, but occasionally they may show increased thirst, diuresis, a trace of jaundice, or lack of appetite and vague intoxication.

It was the object of Whipple, Robscheit-Robbins, and Hawkins to put the Eck fistula liver under the strain of producing blood proteins in an emergency by the stimulus of artificially induced anemia and hypoproteinemia. The animals were given a diet low in protein content but supplemented with ample iron as well as vitamins. Anemia was produced by blood removal. They had hoped also to cause further strain by producing liver injury, but chloroform was found to be well tolerated by the Eck fistula dog. Such animals were able to survive twice as lethal a dose as the control dog without evidence of significant liver injury. It was possible, however, by giving acacia by vein, to cause deposition of this substance in the liver and thereby to impair the function of the liver in respect to hemoglobin production.

In such animals the effects on hemoglobin production of a variety of substances were tested. These included the feeding of liver, iron, and digests of casein, as well as the injection of colloidal iron. Although the results were not always exactly the same, in general they appeared to show that the anemic Eck fistula animal cannot utilize standard diet factors and iron as efficiently as the anemic non-Eck control. The output of new hemoglobin in some instances dropped to as much as one fourth of normal. Similarly in such animals the production of plasma proteins was impaired, dropping at times to even as little as one tenth of normal.

In the interpretation of these investigators, these data indicate that the liver is concerned directly or indirectly with the production of new hemoglobin. This may be accomplished by the liver contributing to the fabrication of hemoglobin by means of the mobile plasma proteins which to a large extent are derived from the liver. The blood plasma may supply proteins to the red cell which may modify and perhaps aggregate them with parts of the degenerating nuclear material of the red cell to form hemoglobin.

PIGMENTATION IN VITAMIN E DEFICIENCY

It has been known for several years that in weanling rats deprived of vitamin E, a brown discoloration of the uterus develops. A similar but less intense pigmentation occurs in the smooth muscle of the cervix, vagina, oviducts, seminal vesicle, and ureter and also in dystrophic skeletal muscle. It was shown by Martin and Moore (*J. Hygiene* **39,** *643 (1939)*) that the pigmentation of the uterus and seminal vesicles is accompanied by a deposition of small yellow granules in the smooth muscle cells. Mason and Emmel (*Yale J. Biol. Med.* **17,** *189 (1944)*) have added to our knowledge concerning the distribution and significance of these pigment granules.

Martin and Moore made a number of interesting observations concerning the pigmentation of vitamin E deficiency. The discoloration of smooth muscle was shown to develop gradually; after young female rats had ingested a diet deficient in vitamin E for three months, there was a faint yellowish pigmentation of the uterus, while after one year the discoloration was invariably striking. Pigmentation of skeletal muscle was found only in rats which had shown paralysis and even then it was localized in distribution. That the presence of pigment granules did not necessarily interfere with the normal functioning of the organ involved was shown by the fact that animals treated with vitamin E after a comparatively short period of deprivation gave normal litters, although the discoloration of the uterus persisted. The administration of alpha-tocopherol was successful in preventing the deposition of pigment, but it was difficult or impossible, once this material was laid down, to effect its removal. This difficulty of removal may possibly be explained by the observation that the pigment is extraordinarily resistant to all solvents. Granules were still apparent even after the treatment of tissue preparations with 30 per cent potassium hydroxide or 10 per cent nitric acid.

The investigations of Mason and Emmel showed that pigmentation was not restricted to the tissues mentioned above but was found also in the ovaries and testes of vitamin E deficient rats. The pigment granules (or more accurately globules, according to these authors) were shown to escape from the muscle cells of various tissues. Following escape from muscle cells, the pigment was taken up by phagocytes. A portion was widely dispersed via the lymphatic and vascular channels; pigment laden phagocytes were found in the lymph nodes and spleen, and pigment globules in the Kupffer cells of the liver. A large amount appeared to have remained in the tissues in which it was formed, in phagocytes which had lost their motility. In the ovaries and testes these phagocytes tended to fuse into giant cells. Treatment with alpha-tocopherol had, at best, a slow and limited effect on the phagocytes and giant cells and on the pigment which they contained. The globules showed great resistance to strong acids, strong alkalies, fat solvents, and oxidizing agents. They were poorly delineated by routine histologic stains such as hematoxylin and eosin but showed a specific affinity for basic fuchsin and, to some extent, for iron hematoxylin. The authors suggested that they might represent an exceptionally stable protein-lipid combination.

From their experimental data, Mason and Emmel were led to the conclusion that the pigment material represented either (1) a normal metabolite which was utilized or degraded with difficulty by cells deprived of vitamin E or (2) an abnormal metabolite which arose because of a deficiency of this vitamin. The fact that normal ovaries contained an indistinguishable pigment, although in much smaller amounts, appeared to favor the alternative that it was a normal metabolite. On the other hand, the observation that therapy with vitamin E affected the pigment in ovaries and testes with con-

siderable difficulty and that in skeletal muscle apparently not at all, favored the other alternative, that its formation represented a deranged metabolism.

Mason and Emmel made the interesting suggestion that this pigment might be identical with "ceroid" which is found in the livers and other tissues of rats with liver cirrhosis (*Nutrition Reviews* **2,** *183* (*1944*)). It is certainly not inconceivable that an identical material might arise in muscle because of an abnormality in metabolism (due to vitamin E deficiency) and in liver as an indirect result of a failure of transport (due to choline deficiency). Sufficient data are not at hand, particularly concerning the staining reactions of the substance found in vitamin E deficiency, to settle the matter of identity. In addition to tests of staining characteristics, further knowledge might be gained by a study of dietary precursors. Endicott, Daft, and Sebrell (*Proc. Soc. Exp. Biol. Med.* **57,** *330* (*1944*)) have succeeded in obtaining dietary liver cirrhosis without ceroid in rats by omitting cod liver oil from the experimental diet and have suggested the probability that some substance or substances present in this oil cause the appearance of "ceroid." An extension of this study and an application of a similar technic to the investigation of the pigment of vitamin E deficiency might not only determine the identity or nonidentity of the two substances but might also be of assistance in the elucidation of the metabolic processes involved. As stated by Mason and Emmel concerning the material found in vitamin E deficiency: "Further characterization of this pigment, histologically and chemically, might provide an important clue to some particular cellular enzyme system, or systems, whose physiological integrity depends upon the presence of vitamin E."

LYSOZYME, AVIDIN, AND BIOTIN

Lysozyme is an enzyme belonging to an interesting group of mucolytic enzymes. This group includes the important hyaluronidase (spreading factor) which liquefies hyaluronic acid, a substance partially responsible for holding the tissue cells together. Lysozyme itself lyses certain microorganisms. Laurence and Meyer (*see Nutrition Reviews* **2,** *291* (*1944*)) reported evidence strongly suggesting that lysozyme is a combination of the vitamin biotin and the so-called "antibiotin" avidin. There were two lines of evidence: (1) Lysozyme and avidin were encountered together in natural sources and partially purified preparations in somewhat constant proportions; (2) biotin, added to preparations containing both lysozyme and avidin, greatly enhanced the lysozyme activity.

Alderton, Lewis, and Fevold have now investigated this problem further (*Science* **101,** *151* (*1945*)) and fail to find any chemical relationship between lysozyme and avidin. They had at their disposal the recently reported crystalline lysozyme (Alderton, Ward, and Fevold, *J. Biol. Chem.* **157,** *43* (*1945*)) and many other preparations from egg white containing various proportions of avidin and lysozyme. Each preparation was analyzed for its concentration of lysozyme, biotin, free avidin, and avidin combined with biotin. Furthermore, the effect of biotin addition on the lysozyme activity was measured.

Crystalline lysozyme contained almost no biotin—only one-millionth the amount demanded by the hypothesis that biotin is its prosthetic group. The avidin content was vanishingly small, and the addition of biotin did not increase the lysozyme activity. Likewise, in crude avidin preparations containing both lysozyme and free avidin, as well as in a purified avidin preparation almost devoid of lysozyme (less than 0.1 per

cent), the addition of biotin failed to enhance lysozyme activity. To test further for possible enhancement, both natural and synthetic biotin in large and small concentrations were used. Furthermore, biotin and lysozyme were incubated together for as long as eighteen hours before testing, and live as well as killed microorganisms were used as test material. In all cases the results were negative. Woolley and Longsworth (*J. Biol. Chem.* **142,** *285 (1942)*) also have obtained a highly purified avidin preparation devoid of lysozyme activity.

It thus seems clear that lysozyme cannot consist of an avidin-biotin complex. The discrepancy between these findings and those of Laurence and of Meyer remains to be explained. Alderton *et al.* suggest that avidin and lysozyme tend to go into the same chemical fractions during partial purification because they both have alkaline isoelectric points (10.0 and 10.8 respectively).

It is more difficult to explain the difference in the results obtained on addition of biotin. The method used for measuring lysozyme activity was not the same in each case. Alderton *et al.* measured the rate of decrease in turbidity of a suspension of M. lysodeikticus buffered at pH 6.2 (method of Boasson, *J. Immunology* **34,** *281 (1938)*). Meyer allowed the enzyme to act on the same organism but at a more acid pH (about 4.4). The solution was essentially unbuffered, the potassium dihydrogen phosphate which was added having almost no buffer capacity at this pH. At an acid pH, lysozyme is more active, but the organisms do not dissolve. Therefore, alkali was added after the enzyme action and the smallest amount of enzyme which would produce complete lysis under these conditions was measured. It seems possible that this difference in analytical technic may explain the discrepancy in results. Conceivably, the solution of biotin may have contained acid or base which brought the test system to a more favorable pH. Another possibility is that the biotin content of the test organisms themselves might be a factor. However, the organisms of Alderton *et al.* contained only 0.93 microgram of biotin per gram dry weight.

In any event it would appear that biotin is yet to be identified as the prosthetic group of an enzyme. Meanwhile, lysozyme and related ferments, on the one hand, and biotin on the other, will continue to attract well deserved research attention.

NUTRITIONAL REQUIREMENTS FOR WORK

Justus Liebig ("Animal Chemistry," translated by William Gregory, *James M. Campbell and Co., Philadelphia, p. 72 (1843)*) advanced the concept that the breakdown of protein was the source of mechanical power in the animal organism. A corollary of this hypothesis was that protein requirements increased with increased work. This erroneous idea of protein requirements was disproved by Professor Chittenden ("Physiological Economy in Nutrition," *Stokes, New York (1905)*), who demonstrated that a group of athletes could reduce their nitrogenous metabolism to as low a level as could a group of professional workers "with not only maintenance of health and strength, but with a decided increase in their muscular power."

Such an outstanding historic example of the failure of work to increase the requirements of man for a food substance should have served as a caution in hypothesizing a necessarily increased vitamin requirement of persons engaged in physical labor. However, there are some who advocate the general distribution of various vitamin preparations to industrial workers (*see* The Nutrition Front, New York State Joint Legislative Committee on Nutrition, *Legislative Document no.* **64,** *Albany (1943)*).

In a critical review of the effects of hard

physical work upon nutritional requirements, Forbes (*Milbank Memorial Fund Quart.* **23,** *89 (1945)*) discussed especially the vitamin requirements in work. He points out that the requirements may be (1) practically unaltered by work, (2) increased in proportion to the work, or (3) increased in proportion to the displacement of the equilibriums of the body. The pattern characteristic for a vitamin may be investigated by inquiring as to the final effect of the deficiency or as to the influence of hard work on the rate at which the deficiency appears. An examination of existing evidence led Forbes to conclude that "the indications are that light or moderate or even hard physical work add primarily to the caloric requirements and increase the needs for protein and vitamins A, D, C, and K little if at all. The need for the B-complex may be increased somewhat but probably less than in proportion to the extra calories until the work becomes hard or exhausting, when it almost certainly rises considerably." (*See Nutrition Reviews* **1,** *227 (1943)*; **2,** *115, 140, 334 (1944)*; **3,** *5 (1945)*.) Further, Forbes states that "nothing is known of the extra requirements, if any, for the kind of work that the average industrial worker performs, namely, work which is physically easy but nervously or emotionally tiring."

This review is concluded with the interesting suggestion that the doing of moderate work may improve the nutritional status of a man on a diet limited in quality but not in quantity because of the larger amounts of food which the subject will consume. Such a view explains the observations of Lind (quoted by Hess, "Scurvy, Past and Present," *Lippincott, Philadelphia, p. 57 (1920)*) that the slothful sailor was more often stricken with scurvy than was the active individual and that physical exercise tended to bring about a cure of scurvy.

THE HEMOGLOBIN LEVELS OF COLLEGE WOMEN

Chaptal ("Chymistry Applied to Agriculture," *First Am. Ed., Hilliard, Gray, and Co., p. XX, Boston (1835)*) observed that "all sciences have a natural course from which they never deviate: they begin by collecting and proving facts; and when these facts are well established, they compare them with each other, and deduce from them principles of general application." The science of nutrition is now in the state of "collecting and proving facts." It is, therefore, pertinent to inquire with Chaptal, "will a fact observed in one place be constantly reproduced in another"?

Two recent additions to our collection of facts concerning hemoglobin levels in young women are worth noting. The first of these (Ohlson and co-workers, *Am. J. Physiol.* **142,** *727 (1944)*) is a carefully planned cooperative study from the nutritional laboratories of Iowa State College, Kansas State College, Oklahoma A. and M. College, and the Universities of Minnesota, Wisconsin, and Nebraska. Data are reported on 4550 young college women from 16 to 30 years of age with 90 per cent of the subjects within a 17 to 24 year age group. The determinations were made on capillary blood by the acid hematin method except at one school (Nebraska) where an oxyhemoglobin procedure was applied to venous blood. Erythrocytes and packed cell volumes were also estimated. The sample is described as consisting of "essentially normal" young women. Although no reference is made to racial stock or color these are not likely to affect the results unduly. The latter factor may, under certain conditions, however, be of considerable importance (Dill, Wilson, Hall, and Robinson, *J. Biol. Chem.* **136,** *449 (1940)*; Munday and co-workers, *Am. J. Dis. Child.* **55,** *776 (1938)*). The data may be summarized by indicating the means and standard deviations for the entire series:

hemoglobin, 13.4 ± 1.16 g. per 100 ml. of blood; erythrocyte counts, 4.56 ± 0.38 million cells per c.mm.; mean cell volume, 40.9 ± 2.63 per cent. The maximum range of hemoglobin variation was 8.5 to 17.5 g. It was concluded that "normal standards for hemoglobin concentration, red cell counts, and cell volumes must be interpreted as a range of values which is wider than the ranges recorded by earlier studies."

The hemoglobin data on a series of 864 college women from 16 to 22 years of age at the Royal Victoria College, McGill University, are presented by Pedley (*Canad. Med. Assn. J.* **51,** *351* (*1944*)). These determinations were carried out by the oxyhemoglobin procedure on capillary bloods. The results are not well presented, but it may be calculated that the mean of these values was approximately 13.7 g. per 100 ml.

Such close agreement with the "mean of hemoglobin values reported for strictly normal individuals" (13.8 g. per 100 ml. for the adult female) (Myers and Muntwyler, *Ann. Rev. Biochem.* **9,** *304* (*1940*)) might lead one to suppose that our "facts are well established." The Report of the Traumatic Shock Committee of the Medical Research Council (*Brit. Med. J.* **1,** *209* (*1943*)), however, reminds one that hemoglobin may exist in no less than four different forms in the blood of healthy individuals and under special conditions these inactive forms may rise to appreciable percentages of the total hemoglobin. Some of these inactive forms are not converted to the hemoglobin derivative which is measured by certain widely used procedures. Hence, it is only when the same method has been employed by different investigators that one may properly compare results, and determinations by certain methods may still give but an approximation of the total hemoglobin concentration as measured by the determination of iron in the washed red cells.

Hemoglobin levels are almost universally used (correctly or not) as a measure of nutritional status, and it is sometimes hypothesized that cases with "mild or slightly subnormal blood levels" (Wiehl, *Milbank Memorial Fund Quart.* **19,** *45* (*1941*)) are of particular interest in this connection. Hence, standards must be set up with meticulous care with the known variables constantly in mind. In order that "a fact observed in one place (can) be constantly reproduced in another," each worker must define his sample as to age (physiologic and chronologic), color, sex, apparent state of health, altitude, method of collecting the blood, method used in the determination, and any other factors which might affect the results. Further, the presentation and interpretation of such data must be along sound statistical principles. Thus the conclusion of Pedley that "in view of the fact that 40 per cent of the girls showed hemoglobin levels between 14 and 16 gm. per 100 cc. of blood, it is suggested that a 'good,' if not 'optimum,' level of hemoglobin in women should be set at about 15 gm. per 100 cc." is indefensible. Such arbitrary attempts to set "normal" levels are based upon the hypothesis that nothing less than maximum is good. Fowler and Barer (*Am. J. Med. Sci.* **201,** *642* (*1941*)) have demonstrated that many healthy young women have a hemoglobin level which cannot be raised by therapeutic measures to 14 g. per cent. Obviously, hemoglobin concentrations, like all physiologic attributes, must be thought of in terms of frequency distributions over a range compatible with health. All persons who fall into the lower part of that range must not be assumed to be subnormal or "suboptimal."

THE NUTRITIVE QUALITY OF GOAT'S MILK

It has been estimated that there are over 1,000,000 milk goats in the United States and that the retail value of the goat's milk produced here exceeds $100,000,000 annually

(*see* Gamble, Ellis, and Besley, *U.S.D.A. Tech. Bull. no.* **671,** *March (1939);* Holmes *et al., New Engl. J. Med.* **232,** *72 (1945)*). Although goat's milk is not widely used for infant feeding in this country it is advocated for use in some artificially fed infants who are sensitive to cow's milk. From Europe, where this product has been more generally used in infant feeding, there have appeared a number of reports of "goat's milk anemia" which is seen in children fed exclusively on this food. Rominger, Meyer, and Bomskov (*Z. f. d. ges. exp. Med.* **89,** *786 (1933)*) and György (*Z. f. Kinderh.* **56,** *1 (1934)*) have presented evidence which they interpreted as indicating that this anemia is not a simple iron deficiency disease but is due to a deficiency of the extrinsic factor of Castle. An examination of some of the more recent studies bearing upon the nutritive qualities of goat's milk would appear profitable.

Numerous workers in this country have attempted to define the nature of the deficiency in goat's milk responsible for the anemia which develops in experimental animals which are fed exclusively upon this product. Thus, Orten and Smith (*Yale J. Biol. Med.* **8,** *637 (1936)*) demonstrated that rats did not become anemic when maintained from the third to the thirteenth week of age on a diet of goat's milk supplemented with iron and copper. Similarly, the anemia produced by restricting rats to a diet of goat's milk was cured solely by the addition of iron and copper (Kohler, Elvehjem, and Hart, *Am. J. Physiol.* **113,** *279 (1935)*). Identical results have been reported by Gamble, Ellis, and Besley (*loc. cit.*). These latter workers found that weight gains by rats, goat kids, and healthy human babies maintained on goat's milk for a period of ninety days were proportional to the total energy content of the milk consumed. Kohler *et al.* reported that rats maintained on a mineralized goat's milk failed to grow normally unless the diet of the lactating goat was of good quality.

A comprehensive study of the composition and properties of goat's milk as compared with cow's milk was made by Gamble, Ellis, and Besley, the findings of which have been summarized by Besley (*Am. J. Pub. Health* **30,** *182 (1940)*). The study was made on pooled milk supplied by the Beltsville herd of the Bureau of Animal Industry. The average percentage composition of the goat's milk was as follows: water, 88.29; lactose, 4.55; fat, 3.5; protein, 3.10; ash, 0.79; calcium, 0.114; and phosphorus, 0.098. The iron content was 0.068 mg. per cent and the copper content 0.053 mg. per cent. The mean content of vitamin A was 2500 I.U. per 100 g.; that of vitamin D was 7.4 I.U. per 100 g. The general composition of the goat's milk was found to simulate closely that of Holstein milk which was produced simultaneously. The chief differences were slightly higher calcium, phosphorus, albumin, and nonprotein nitrogen values for the goat's milk. The fatty acids in the butterfat of the latter were found to consist of somewhat smaller quantities of the unsaturated acids and more of the volatile ones than found in the Holstein milk. Goat's milk showed greater seasonal variation in total caloric value and fat content than did cow's milk.

A later study of the vitamin content of commercial winter goat's milk (Holmes *et al., loc. cit.*) has again revealed the favorable composition of this food. The samples of raw milk examined were from localities within an area of one hundred miles of Amherst, Massachusetts, and were held in a milk refrigerating room until they were 7 days old. The methods employed in the determination of the vitamins were as follows: titration with 2,6-dichlorophenolindophenol for ascorbic acid (Holmes, Tripp, Woelffer, and Satterfield, *J. Nutrition* **17,** *187 (1939)*), microbiologic methods for niacin and pantothenic acid, a fluorometric procedure for riboflavin, and a thiochrome technic for thiamine. The means of the values expressed in milligrams per liter

were: ascorbic acid, 6.5; niacin, 2.96; pantothenic acid, 3.38; riboflavin, 1.25; and thiamine, 0.45. The data are characterized by considerable variability. In particular is this true of the ascorbic acid values which ranged from 0.2 to 14.7 mg. per liter. The relatively low bacterial count of goat's milk is indicated by Holmes *et al.*, Besley, and Gamble *et al.*

It appears, in summary, that the composition of properly produced goat's milk is similar to that of cow's (Holstein) milk, and that experimentally produced "goat's milk anemia" is (as found also from feeding tests with unsupplemented cow's milk) due to a lack of iron and copper. In special cases, however, when the animal's food supply has been extremely limited, anemia may result from poor quality with respect to other nutrients than copper and iron.

THE CAUSE OF TROPICAL ULCER

Tropical ulcer is a disease with a confusing etiology. Many clinical investigators have noted the presence of spirochetes and fusiform bacilli in the lesions, and one authority has attributed the condition to Spirocheta schaudinni. Others believe a diplococcus to be the causal organism. There is one record of the direct transmission of a typical sore to an abraded surface, although most such attempts have been unsuccessful. Despite the part undoubtedly played by an infective agent, there is a widespread and growing belief that the fundamental etiologic or predisposing factor is one of malnutrition. Deficiencies of vitamin A and of riboflavin in the diets of susceptible groups have been noted, while excellent therapeutic results have been reported from intravenous injections of calcium chloride. It appears probable, therefore, that in this disease an interrelationship is involved between a dietary deficiency (or deficiencies) and a susceptibility to infection. For this reason, the subject of tropical ulcer holds considerable interest for us, despite the relative lack of clinical significance of the disease in this country.

Manson-Bahr ("Manson's Tropical Diseases," *William Wood & Co., Baltimore (1940)*) describes tropical ulcer as common in most tropical countries, particularly in those with a hot, damp climate, principally in the jungle. It is described as frequent among the carriers attached to the East African Force during World War I, as the most common cause of disability among laborers in Malaya, and as a terrible scourge throughout the whole of Melanesia. The first sign in previously sound skin is the formation of a bleb. In the course of a few hours the bulla ruptures, exposing an ash-gray, moist slough which rapidly extends in all directions. After a few days the center of the slough begins to liquefy and in the course of a week or longer the slough may be thrown off. In bad cases, muscles, tendons, nerves, vessels, and even the periosteum of the bones have shared in the gangrenous process.

Charters (*Tr. Roy. Soc. Trop. Med. Hyg.* **37,** *205 (1943)*) has reported that tropical ulcer is of especial interest during the present war because of its high incidence among the native troops. He has noted an extraordinary distribution of the disease. Of a total of 570 East Africans admitted to the Eighth (E.A.) Field Ambulance in a six month period, there was only one case of ulcer and even there the diagnosis was doubtful. Of 292 Somali soldiers admitted during the same period there were, in contrast, 143 cases. The Somalis and East Africans were living under identical climatic and other conditions with one exception—they differed in their diet. This difference was because the Somalis disliked and refused certain foods in the official ration which were consumed and enjoyed by the East

Africans. In order to confirm the suspicion that the dietary difference was responsible for the peculiar distribution of the disease, 6 Somalis with tropical ulcer were persuaded to eat the ordinary East African diet. All cases quickly improved.

A study of the composition of the Somali diet revealed that there were adequate allowances of protein, phosphorus, iron, calories, thiamine, and ascorbic acid. There were, however, shortages of calcium, vitamin A, and riboflavin. In place of the estimated daily requirements of 0.6 g., 5000 I.U., and 3 mg., respectively, the actual daily intake was 0.3 g., 900 I.U., and 0.5 mg.

Pure vitamins were not available but a therapeutic test was undertaken using cod liver oil as a source of vitamin A and condensed milk as a source of riboflavin. Calcium was given intramuscularly as calcium gluconate or intravenously as calcium chloride. Tests of coramine and ascorbic acid were included. All patients, including the controls which were given the usual Somali diet with no additions, rested in bed and received dry dressings. Forty per cent of the controls showed improvement on this regimen alone. No additional therapeutic benefit was discernible from the treatment with coramine or ascorbic acid. All of the patients given calcium, cod liver oil, or condensed milk, on the other hand, showed a definite and rapid healing. The benefit derived from the condensed milk was finally ascribed to its calcium content since additional patients, given other foods rich in riboflavin, failed to respond. As a check on the effectiveness of vitamin A, 55 patients were given Abyssinian sweet pepper known to be rich in carotene. Fifty-two of these showed prompt signs of improvement.

Charters concluded that vitamin A is probably the principal element in the etiology of tropical ulcer and that the benefits derived from calcium salts were probably due to their effect on capillary permeability. His rejection of a calcium deficiency as an important factor is based on the fact that during the six months of the test (but not previously) the East Africans were ingesting no more calcium than the Somalis. In the absence of more direct evidence than is presented, it appears that this conclusion rests on a somewhat inadequate foundation. The importance of a dietary factor or factors, however, appears to have been demonstrated and there is basis for Charters' suggestion that the dietary deficiency may have predisposed the susceptible group to a specific infection.

PLASMA PROTEIN AND HEMOGLOBIN IN THE PROTEIN DEFICIENT RAT

Stereotyped patterns of research have yielded vast stores of information and advanced knowledge in many directions. The pure culture, the weight curve of growth, the electrocardiogram, the blood pressure cuff have all been tools of great importance in the progress of medical science. By increasing the points of attack in a study, new dimensions are included and new facts brought to light, even when old methods are employed. Concentrations and proportions have become fixed as a routine manner of expressing changes in many of the biologic constituents of blood and urine. Thus a thought pattern has grown up of levels, of parts per 100 ml. or per liter rather than contents expressed as parts per organism, or parts per fraction of surface area. When both contents and proportions are used to express certain data they may show striking differences. This is well illustrated by the three-dimensional study of plasma protein and hemoglobin in the protein deficient rat (Metcoff, Favour, and Stare, *J. Clin. Invest.* **24,** *82 (1945)*). Instead of reporting levels only they expressed their data in terms of contents related to surface area as well.

Seventy-one male weanling (35 to 40 g.) rats were caged individually and fed a purified diet. They were weighed on alternate days. After a week's standardization, base line determinations of hemoglobin, erythrocytes, and blood volume partitions and plasma proteins were made. The data on blood volume partitions, unit circulating protein, and hemoglobin were relative rather than absolute, owing to inherent limitations in the technics used, though the results remained proportional throughout. Twenty-two rats were employed to study the effects of previous nutritional status upon acute protein deficiency. Half were fed at the control level of 18 per cent casein, half at 8 per cent for one month, then both groups were reduced to 2 per cent casein. After seven days on this regimen the blood studies were repeated. The remaining 49 rats were divided into two groups; one was fed casein at the 18 per cent level, one at the 8 per cent level. The blood studies were done at the outset, and after three and six weeks in all animals. In 10 rats from each group they were repeated after the tenth and fourteenth weeks. In comparison of control and deficient rats those of similar size and weight were used so that the better fed controls were 2 or 3 weeks younger than the depleted rats.

Each rat fed casein at an 18 per cent level gained about 4 g. daily and the group more than tripled the initial body weight whereas those fed at an 8 per cent level gained about 1 g. daily and failed to double their initial weight in one month. When these two groups of rats had their protein reduced to 2 per cent, the previously well nourished ones lost an average of 2.5 g. per day and the previously deficient ones lost slightly less than 1 g. per day. Previously well nourished rats suffered a decrease in the unit circulating protein and contraction of unit plasma volume but no change in the plasma protein concentration. Previously deficient animals did have a decrease in plasma protein concentration. Chronic protein deficiency for ten to fourteen weeks did not change plasma protein concentrations significantly though unit plasma volume was decreased in three to six weeks. The decrease in unit circulating protein first noted at three to six weeks had become significant by ten to fourteen weeks.

Acute protein deficiency led to hemoconcentration (increased hemoglobin concentration plus decreased plasma volume), without change in cell volume. Only the previously well nourished rats showed significant decrease in unit circulating hemoglobin. Chronic deficiency of ten to fourteen weeks duration resulted in a decrease in unit circulating hemoglobin but not in its concentration.

The constancy of plasma protein concentration in the face of dietary protein deficiency is in agreement with most of the cited reports, and presumably depends on the masking effect of hemoconcentration. Decreased unit circulating protein in acute or chronic depletion was in part a manifestation of contraction of plasma volume, and it is probable, as the authors point out, that its chief cause was a decrease in plasma albumin, though fractionation of plasma proteins was not done.

The increased concentration of hemoglobin in rats on the 2 per cent protein diet probably resulted from hemoconcentration. Chronic deficiency for fourteen weeks did not effect any significant change. The decrease in unit circulating hemoglobin in "acute and chronic protein deficiency was probably a function of altered blood volume and induced hypochromia."

Mechanisms by which the changes in plasma protein and hemoglobin are effected in the protein deficient rat are largely unknown. The regulation and priority systems of the organism when the intake does not satisfy demands are speculative. It appears that plasma protein has a high priority; growth suffers first, and then body cells are depleted. Plasma volume shrinks

and concentration remains relatively stable. After a time even plasma protein is reduced. If this goes beyond the stage when these experiments were terminated, edema would probably ensue.

The mechanism of anemia is not known but the authors suggest that a decrease in unit cell volume is a prominent factor in its occurrence in chronic protein deficiency.

This study is important because it utilizes several quantitative measures which permit evaluation of contents as well as levels. Had the data been treated in the routine fashion of expressing levels alone, several important findings would not have emerged. This suggests that a careful reevaluation of much data from the clinic might reveal significant facts when considered in the light of blood volume partitions rather than as levels per arbitrary unit of volume.

THE HUMAN REQUIREMENT FOR TRYPTOPHANE

With the newer knowledge of the amino acids required by man, it is inevitable and essential that we have a better understanding of the quantitative requirements of these substances, as has been developed with the vitamins. The work of Holt and co-workers (*Proc. Soc. Exp. Biol. Med.* **48,** *726 (1941)*), of Cox, Mueller, and Fickas (*Ibid.* **51,** *303 (1942)*), and of Rose (*Proc. Inst. Med. (Chicago)* **15,** *24 (1944)*) have shown that tryptophane is a dietary essential for man as well as for the rat. Subjects maintained on a tryptophane deficient diet have been shown to develop a negative nitrogen balance which can be restored to nitrogen equilibrium by the addition of tryptophane. However, no exact information has been available concerning the minimal tryptophane requirement. A study by Holt, Albanese, Frankston, and Irby (*Bull. Johns Hopkins Hosp.* **75,** *353 (1944)*) seeks to give an answer to this question.

The experiment was carried out in the same manner as earlier studies by these workers. Their basal experimental diet consists of fat, starches, and certain vegetables and fruits selected for their low protein content. These provide approximately 10 per cent of the daily nitrogen requirement. The remaining 90 per cent of the nitrogen requirement is supplied by an acid hydrolysate of casein supplemented with 1 per cent l-cystine. Two normal adult male experimental subjects were given 38 to 43 calories per kilogram per day of such a diet. This furnished 0.1 g. nitrogen per kilogram per day. The subjects pursued a sedentary life during the period of the experiment. Preceding the institution of the experiment, one subject had received a low protein diet for two days, whereas the second had taken his customary diet. In a control period of six days' duration both subjects received the tryptophane deficient diet with a supplement of l-tryptophane in an amount representing 1.5 per cent of the protein moiety.

During a thirty-six day period on a deficient diet without tryptophane supplementation, the subjects lost weight and a negative nitrogen balance developed. These results were consistent with previous observations. At the end of this period, l-tryptophane was added to the diet, at first in a concentration representing 0.5 per cent of the protein moiety and then in quantities corresponding to 1 per cent and 1.5 per cent. Thus 3.1, 6.3, and 9.4 mg. l-tryptophane per kilogram per day were furnished in the respective periods. Each of these periods of supplementation was three days in duration.

On the lowest level of tryptophane supplementation, no appreciable influence on nitrogen balance was noted. On the amount corresponding to 1 per cent of the protein moiety one subject exhibited a positive nitrogen balance but the other showed no

improvement. In this second subject l-tryptophane was required in an amount corresponding to 1.5 per cent of the protein moiety to produce a positive nitrogen balance.

The investigators also measured tryptophane excretion in the urine. They observed that during the control period the subjects excreted from 250 to 400 mg. of tryptophane per day. With the institution of the tryptophane deficient diet, the urinary tryptophane fell sharply, remaining below 250 mg. per day. When the lowest level of tryptophane supplementation was given, one of the 2 subjects responded by a return of tryptophane excretion to the normal level. In the second subject it was only at the second level of tryptophane supplementation that a return to the normal level of tryptophane excretion occurred.

These data would suggest that the tryptophane requirement may be between 6 and 9 mg. per day or between 3 and 6 mg. per day, depending on what criteria one follows. The higher figure is suggested by the amount required to produce a restoration of nitrogen equilibrium whereas the lower figure depends on the excretion of tryptophane in the urine. There is known to be a lag in establishing nitrogen equilibrium. This would account for the fact that a higher level of tryptophane seemed to be required to produce a return of nitrogen equilibrium as compared with the quantity which was necessary to produce a change in urinary tryptophane excretion. The value of these studies would have been much greater had circumstances permitted the investigators to carry out longer supplementation periods.

PREVENTION OF RICKETS BY SINGLE LARGE DOSES OF VITAMIN D

It has long been known that a single large dose of either vitamin A or vitamin D can be used effectively as a prophylactic measure against the respective deficiency diseases in the rat. For some years also there have been reports that infants can be successfully protected from rickets for the whole of one winter by a single large dose of vitamin D. Some new clinical observations supporting this view, and a discussion of such prophylaxis, are put forward by Krestin (*Brit. Med. J.* **1**, *78* (*1945*)).

Krestin had reported previously that in 1944 rickets still presented a problem in infant entrants to wartime nurseries in Preston (in the north of England). Severe or advanced rickets in these infants was rare, but more than one third of them under the age of 2 years had early or mild rickets, and the distribution of free cod liver oil for the infants before their admission to the nurseries had not noticeably reduced the incidence.

In the present report a clinical trial was made with full term infants and children from 2 months to 3 years of age, who showed no radiologic or clinical evidence of rickets on admission to the nurseries during the fall of 1943. A single dose of 7.5 mg. of calciferol (vitamin D_2), having an activity of 300,000 I.U., was given to each infant, which was followed by clinical examination each month, and by radiologic study at the end of six months. Through this period no other vitamin D preparation was given, and no exposure to ultraviolet radiation occurred. The group of infants was compared with a control group receiving no vitamin D, and with another group receiving graded doses of cod liver oil.

Of 16 infants less than 6 months of age, 14 remained free of rickets throughout the six month period of observation, and only one out of 121 between the ages of 6 months and 3 years developed any signs of rickets. This result was better than that obtained with a daily dose of cod liver oil supplying

approximately 1500 I.U. of vitamin D. With this dosage 25 out of 28 infants in the younger age group were fully protected, as were 138 out of 146 in the older age group. Thus additional evidence has been secured of the effectiveness of this method of prophylaxis. It is a method which must appeal to the pediatrician, since he can use it at an office visit or in his patient's home and feel assured that full protection against rickets has been afforded. Krestin administered the dose dissolved in 1 ml. of peanut oil given by spoon after a milk feeding. It was readily accepted, and the only ill effect noted was that it was vomited by one infant.

In his discussion of this prophylactic method, Krestin stresses that no untoward results of single large doses of the order used by him have ever been reported, and that much larger doses have been used without ill effect. He suggests that when a child is first seen in the late winter or spring, one dose of 7.5 mg. is probably sufficient for protection until the following winter; when the child is first seen in the fall or early winter, the dose should be repeated after three months. His observations were made upon healthy full term infants, and he emphasizes that for premature infants, and those which are recovering from marasmus or an acute illness, larger or more frequent doses may be required.

THE AVERAGE DIET OF A SOUTHERN COUNTY

Comprehensive nutrition surveys may serve (1) to reveal the nature of nutritional problems (in the broadest sense) within a population, and (2) as an approximate test of the validity of various standards—dietary, laboratory, and diagnostic. The interpretation of such findings in terms of physiologic concepts must be guarded, for as Bigwood ("Guiding Principles for Studies on the Nutrition of Populations," *League of Nations Health Organization, Geneva (1939)*) has aptly written, "... no food consumption study can attain to that level of strict accuracy that might, at first sight, be hoped for." Recognition of this fact does not detract from the usefulness of such data in framing nutrition policies and following trends of the dietary habits of a region. This useful purpose of nutrition surveys is oftentimes overlooked in the arguments over their implications for physiology.

A report based primarily upon the dietary findings in a comprehensive nutrition survey of a second North Carolina county (*see Nutrition Reviews* **3,** *106 (1945)*) has appeared (Milam and Darby, *South. Med. J.* **38,** *117 (1945)*). During the year ending June 30, 1944, this study was made of the rural population of a county in which cotton and hosiery mills provided employment for many of the rural people. Data were collected on 959 individuals in 200 families who were carefully selected as representative of the rural population. The allotment of the families comprising the sample was determined by color and employment class (farmer, mill worker, tradesman, and miscellaneous) of the family head. Data on each subject were based upon a physical examination (including observation of the eyes with a slit-lamp), a seven-day food intake record, and the analysis of a 25 ml. sample of blood for hemoglobin, packed cell volume, erythrocyte count, plasma carotene, vitamin A, ascorbic acid, albumin, and total protein.

Data from the seven-day food intake records on 702 persons were tabulated into eight subgroups according to color, sex, and age (persons over 15 years of age were classified as adults). The intake by season is presented for the adult white group. The fact that the intakes for the four seasons are based upon different small groups of the sample limits somewhat the value of the presentation; however, certain definite and

reasonable trends in the foods consumed are obvious.

A table presents the average individual intake of the thirty-seven foods for adult white men and women by season and a ratio comparison of the sexes. Another table gives the average daily individual intake of the same items for white and Negro children and Negro adults. It is not clear whether these data were extrapolated for the whole year from the single week's diet record or were averaged from four seasonal records. (The former procedure was followed—Ed.)

The white women consumed only about 80 per cent as many calories as did the white men. Their intake of an individual food definitely exceeded that of the men only in citrus fruits. In a comparison of the 27 adult Negro men with the 124 adult white men, the former ate more bread, potatoes, and greens and less milk, eggs, and fruits than the latter, but on the whole the intake of the 2 races compared favorably. The intake by the Negro woman more nearly approximated that of the Negro man than did that of the white woman with respect to the white man.

A table giving the percentage of persons who were "in the habit of eating" each of the thirty-seven food items reveals that milk was consumed by 97 per cent of the whites and 98 per cent of the Negroes, eggs by 85 and 83 per cent, legumes by 91 and 95 per cent, corn bread by 60 and 56, and tomatoes 78 and 67 per cent respectively. Both races had about the same frequency of consumption of butter (81 per cent), sweet potatoes (30 per cent), fat pork (72 per cent), chicken (55 per cent), beef (43 per cent), and citrus fruits (50 per cent). Fewer Negroes than whites ate enriched bread, ice cream, cheese, dried fruits, yellow vegetables, Irish potatoes, and corn. More Negroes than whites ate fish, molasses, nuts, and green leafy vegetables. These figures are based upon a single one week food intake record per person and therefore are minimal.

The general impression is gained that this particular group is well fed. The diet as revealed by the average daily intake of the adult white male "is in striking contrast to the fat pork, grits, and molasses monotony, so often said to characterize southern rural diets." The caution which the authors enjoin in consideration of their averages should be stressed because with relatively small samples and with considerable spread in distribution, a mean may be misleading. However, the brief statement of the physical state and the laboratory findings would bear out the impression of a rather well nourished population.

The seemingly low calory level recorded—over-all average of 2000 for whites and 1800 for Negroes with the respective adult males eating 2500 and 2200 calories—is in agreement with that found in other similar surveys (Milam and Anderson, *South. Med. J.* **37,** *597 (1944);* Youmans, Patton, and Kern, *Am. J. Pub. Health* **33,** *58 (1943); Nutrition Reviews* **1,** *302 (1943); Ibid.* **3,** *106 (1945)*). Again, this reemphasizes the need for a more careful consideration of standards of caloric requirements. The intake of ascorbic acid (when allowance is made for the fact that it was calculated only in raw foods and tomatoes) appears adequate, as does that of vitamin A, protein, fat, and iron. The mean intake of thiamine was approximately 1 mg. per day which sufficed to give a favorable thiamine nonfat calory ratio of 0.9. The recorded riboflavin intake averaged 1.4 mg. with a ratio of riboflavin to total calories of 0.8, interpreted to be "considerably above the safety level of 0.5." The mean daily intake of niacin was 9 mg., only three fifths of the recommended allowance, but within the range previously calculated for two similar surveys (Dann, *Federation Proceedings* **3,** *159 (1944)*). About 5 per cent of the individuals had calcium intake levels under 0.3 g., although the over-all average was satisfactory.

In the brief discussion of the effects of this diet on the individuals it was concluded that the most prevalent abnormalities

definitely attributable to malnutrition were obesity, underweight, and low hemoglobin concentration in the blood. The opinion was expressed that it was unlikely that this latter finding could be attributed to a dietary iron deficiency. These problems have not received a great deal of attention from most nutritionists in the past and it would appear profitable to give greater emphasis to these defects in practice.

The authors state that "the specificity of several of the 'signs' of malnutrition was denied by the finding of other more probable causes for them in most of the individuals where they were noted, and further by the fact that they were as frequent in individuals having the higher levels of intake in the specific dietary nutrient (vitamin). It was concluded that in populations with the relatively high nutritional level of the one investigated here, the reported signs of early deficiency states are not specific for malnutrition and hence cannot alone be used to delimit the prevalence of malnutrition. . . . The data do not support the thesis that any serious malnutrition exists within the population surveyed." An evaluation of this argument must await the more detailed report of the physical findings.

BIOTIN DEFICIENCY IN THE RHESUS MONKEY

Acute biotin deficiency was produced in the monkey some years before biotin itself was characterized. Several groups of workers were studying the so-called "egg white injury," now known to be a deficiency of biotin caused by the combination of the biotin in the intestinal contents with the protein of raw egg white in such a way that it cannot be absorbed. The earliest observations were made on rats, but Lease, Parsons, and Kelly (*Biochem. J.* **31,** *433 (1937)*) extended our knowledge to other species, including the monkey, Macacus rhesus. With an artificial diet containing for the first few days 20 per cent, then 30 per cent of egg white, and an aqueous alcoholic extract of wheat germ and yeast as a source of the vitamin B-complex, 2 monkeys showed roughening and thinning of the hair, and some bald spots after two months. At this time the amount of egg white in the diet was raised to 40 per cent. The eyelids and nose became abnormally reddened, and the monkeys were listless and sat huddled up. These signs although not extreme were considered analogous to the egg white injury seen in rats, and a test was therefore made by steaming the egg white before mixing it in the diet; this procedure was known to make it innocuous to rats. The redness of eyelids and nose rapidly disappeared and posture and activity both improved, but hair loss continued. After eighteen days on the steamed egg white diet, a daily dose of an aqueous extract of liver was given to the monkeys, following which a new growth of hair appeared.

In recent years the hair loss has again been noted as a result of biotin deficiency of Waisman and Elvehjem (*J. Nutrition* **26,** *361 (1943)*). Their monkeys were given a basal diet of sucrose, purified casein, salt mixture, corn oil, and cod liver oil, with daily supplements of thiamine, nicotinic acid, riboflavin, pyridoxine, pantothenate, choline, p-aminobenzoic acid, inositol, and ascorbic acid. Eleven rhesus monkeys were used in the experiments on biotin. Some of them received a daily supplement of 20 micrograms of biotin in the form of a concentrate from the beginning, others only after nutritional failure on the basal regimen was evident. The addition of the biotin to the other supplements listed above did not prevent nutritional failure, which was attributed to a deficiency of "folic acid" and was seen as anorexia, weight loss, and leucopenia. While the weight changes in the

monkeys were not affected by the biotin, there was convincing evidence that this factor is needed for the maintenance of normal fur. Monkeys which were given the basal diet and supplements together with a source of "folic acid" lost their coats in two to seven months, and the daily dose of 20 micrograms of biotin then begun was followed by a new growth of the coat which was completely restored in several animals in four months.

Waisman, McCall, and Elvehjem (*J. Nutrition* **29,** *1 (1945)*) have extended their earlier observations by producing biotin deficiency in rhesus monkeys (Macaca mulatta) by three methods. Chronic biotin deficiency was obtained by feeding the diet described above together with various liver preparations as a source of "folic acid." The biotin content of these extracts was assayed microbiologically and hair loss was observed to follow the ingestion of less than 10 micrograms of biotin daily in the "folic acid" source. When the "folic acid" source supplied more than 12 micrograms of biotin daily no fur changes occurred in twenty-eight months.

Acute biotin deficiency was produced in two ways: by the inclusion in the basal diet either of egg white or of sulfa drugs. The diet containing egg white produced signs identical with those described by Lease, Parsons, and Kelly, but the feeding period before they appeared varied from twenty-one days to more than one hundred five days in different monkeys. The dermatitis was more severe than that produced in the chronic deficiency experiment. The same acute deficiency was also produced by adding 3 per cent of sulfasuxidine to a diet consisting of the basal diet described above with the addition of 1 per cent of a liver extract. It had been shown that without the sulfasuxidine this diet did not cause nutritional failure. The effect of the sulfasuxidine could be counteracted by giving 20 micrograms of biotin methyl ester daily. The authors conclude that some biotin is synthesized by the microorganisms of the intestinal tract, but that the amount made available in this way is insufficient for the monkey's needs. The possibility must also be considered that other types of diet, approximating a diet of natural foodstuffs, might permit the synthesis of larger amounts of biotin by increasing or altering qualitatively the intestinal flora.

CARBOHYDRATE METABOLISM IN DIABETES

The discovery that the islets of Langerhans can be selectively destroyed by the injection of alloxan (Dunn, Sheehan, and McLetchie, *Lancet* **I,** *484 (1943)*) has introduced a potent new tool for the study of diabetes, since animals can be rendered diabetic without surgical removal of the entire pancreas and the consequent loss of functions other than the formation of insulin. Stetten and Boxer (*J. Biol. Chem.* **156,** *271 (1944)*) have used this technic to advantage in studying the carbohydrate metabolism in diabetes with the aid of deuterium.

In earlier papers (*J. Biol. Chem.* **155,** *231, 237 (1944)*) these authors had shown that glycogen synthesized *in vivo* from smaller molecules can be labeled by enriching the body fluids with heavy water. By isolating the liver glycogen and determining its proportion of deuterium in comparison with the proportion in the body water, it was possible to calculate what fraction of the glycogen arose from nonisotopic dietary glucose, and what proportion from isotopic molecules, smaller than glucose, formed within the body. In the present paper this method is applied to the study of the source of the urinary glucose in diabetes.

Of a number of rats injected with alloxan, one which survived and developed the signs

of chronic diabetes was chosen. It was maintained on a high carbohydrate diet, containing 60 per cent of starch and no fat. After the injection of alloxan it developed a transient ketonuria and lost about 25 per cent of its body weight, depleting chiefly the depot fat. The body weight then fluctuated about a fixed level, while the signs of diabetes persisted—secretion of a large volume of urine containing from 3 to 9 per cent of glucose.

Ten days after the alloxan treatment, sufficient heavy water was injected to raise the proportion in the body water to 1.8 per cent, and the drinking water was replaced by 2.5 per cent D_2O. The urine was collected frequently and analyzed for glucose, and the sugar isolated as the penta-acetate. The deuterium content of the penta-acetate and of the water simultaneously excreted was determined. Fifty-six hours after the injection of the heavy water the rat was killed and the glycogen and fatty acids isolated both from the liver and the carcass, for determination of the deuterium content.

During the period of observation the rat excreted 14.2 g. of glucose, with a deuterium content indicating that about one quarter of the glucose had been synthesized from smaller fragments than hexose molecules within the body, while the remainder was of dietary origin. The liver glycogen was found to contain a higher proportion of deuterium than was earlier found in normal rats given heavy water, but the carcass glycogen contained the same proportion as in the normal rat. The most striking finding was that the liver fatty acids contained much less deuterium than in the normal rat, showing that fatty acid synthesis had occurred more slowly. The authors calculate that the synthesis was reduced to about 0.1 g. per day, or only 5 per cent of the normal figure. They also calculate that the difference between the amount of glucose used for fatty acid synthesis by the normal and by the diabetic rat is of the same order as the amount excreted in the urine by the diabetic animal.

Thus Stetten and Boxer offer clear evidence of impairment of fatty acid synthesis from carbohydrate precursors in the diabetic animal. In spite of the abundance of glucose within the body, it is not utilized for fat synthesis either because of failure in the system which mediates the synthesis or because the carbohydrate intermediate involved in fat synthesis is not maintained at a sufficiently high concentration at the place of synthesis.

This new metabolic finding in diabetes invites a discussion of other recent interpretations of the action of insulin and the mechanism of fat synthesis. Previous metabolic disturbances noted in diabetes include the finding of Bueding, Wortis, and Fein (*Am. J. Med. Sci.* **204,** *838 (1942)*) in which a failure in pyruvate formation from glucose was indicated, and of Rice and Evans (*Science* **97,** *470 (1943)*) who indicate that insulin is necessary to maintain pyruvate oxidation in muscle. These findings might suggest that failure of fat formation in diabetes is due to lack of a necessary carbohydrate intermediate. On the other hand, Kaplan and Greenberg (*J. Biol. Chem.* **150,** *479 (1943)*) and Sacks (*Science* **98,** *388 (1943)*) implicate a role of insulin in the formation of "energy-rich phosphate." Since the formation of fat from carbohydrate requires energy, and the modern concept of transfer of carbohydrate energy is through "energy-rich phosphate," a failure of this energy source might also explain a lack of fat formation in diabetes.

More specific theories of insulin action and the metabolic upset in diabetes await a better understanding of the mechanism of fatty acid formation. The exclusive occurrence in nature of fatty acids with an even number of carbon atoms supports the belief that a two-carbon carbohydrate is primarily involved. The isotope experiments of Rittenberg and Bloch (*J. Biol. Chem.* **154,** *311*

(*1944*)) clearly demonstrated a synthesis of fat from acetic acid in the rat, evidently by successive condensation of two-carbon units. The recent isotope dilution experiments of Medes, Weinhouse, and Floyd (*Ibid.* **157,** *751* (*1945*)) show that a large proportion of acetic acid added to kidney slices is transformed to ketone bodies. Whether this reaction might constitute an initial step in the synthesis of fatty acid remains to be proved.

All these studies indicate a focusing of attention on a role of insulin in the transformation of carbohydrate to fat.

HOST NUTRITION AND NATURAL RESISTANCE TO INFECTION

Much has been written regarding the role of nutrition and natural resistance to infection. Various phases of this subject have been discussed in these pages (*Nutrition Reviews* **1,** *298* (*1943*); *Ibid.* **2,** *241* (*1944*); *Ibid.* **3,** *69*, *78* (*1945*)). Certain experimental studies are open to criticism for several reasons. Thus in some instances the experimental animals were really debilitated ones whose lives were already in jeopardy from the consequences of the produced deficiency. Again, many of the published reports do not include records of controls left uninfected. As a result it is difficult to decide whether the animals died as the consequence of the infection or because death was hastened by the infection, and it is often impossible to judge whether the animals would have died anyway from the results of the deficiency alone independently of the infection. From the point of view of the care taken in setting up the experiment, the study by Schneider and Webster (*J. Exp. Med.* **81,** *359* (*1945*)) is as refreshing as its cautiousness in interpretation is impressive.

The study was carried out on various pure inbred strains of mice entirely free from Salmonella enteritidis infection, one of the "mouse typhoid" agents. These strains of mice differed widely and predictably in their response to infection with this pathogen, which is a "natural" one for mice and is responsible for epizootic disease in wild as well as laboratory populations. It has been found to lend itself well to controlled infection technics and when introduced into the gastrointestinal tract, the "natural" route, incites an infection which reproduces the disease as it occurs spontaneously. Painstaking precautions were followed by the investigators with respect to the environment of the animals and the general conditions of the experiment.

From the viewpoint of nutrition, it was proposed to compare the resistance of mice to infection when receiving a "normal" diet as compared with a synthetic one. The decision as to what might be regarded as a normal diet of mice is naturally not a simple one, but for reasons stated, the authors concluded that a diet consisting in the main of a cultivated cereal 2 parts, and dried whole milk 1 part might be regarded as "normal." On the basis of physiologic activities such as growth, fertility, fecundity, and ability of the females to rear their young to weaning, as well as growth of the progeny and the resistance of the progeny, it was concluded that when the cereal in this diet was whole ground wheat, the diet was superior to one in which potato or whole yellow corn, whole ground rye, whole ground oats, or whole ground rice replaced the whole ground wheat. For comparison with this a synthetic diet was chosen consisting of casein, glucose, salts, l-cystine, beta-carotene, viosterol, tocopherol acetate, and the following water soluble vitamins: thiamine, riboflavin, pyridoxine, niacin, choline, p-aminobenzoic acid, and inositol. (Although calcium pantothenate is not listed in the dietary table,

mention of this vitamin elsewhere and the implied assumption that all the known vitamins except biotin and "folic acid" were included in this diet, would suggest that its omission is only a typographical one.)

Comparison of survival rates of infected animals indicated significantly greater survival for the animals receiving the "natural" diet as compared with those receiving the synthetic one. Various factors, such as the method of sampling and the size of the infecting dose did not influence this conclusion. A preliminary search for the constituents responsible for the greater resistance on the natural diet was made by adding whole wheat or dried milk to the synthetic diet to the extent of 66 per cent of the latter. The results were clear in that the greater natural resistance of mice subsisting on the natural diet was manifestly due to the whole wheat that it contained. Dried milk contributed nothing to the beneficial effect. The benefit appeared not to be related to weight, for the animals receiving the synthetic diet plus dried milk actually were heavier than the group receiving the synthetic plus whole wheat diet. Nor did the superiority appear to be due to an increase in protein since, for example, the synthetic plus whole wheat diet had a lower protein content than the synthetic plus dried milk; neither was it due to a qualitative difference in the protein since the protein of dried milk is generally regarded as more complete than that of wheat. As to the influence of vitamins, it is natural to wonder whether biotin or "folic acid" might have played a part. The whole wheat contributed only one-third as much biotin to the basal diet as did the whole dried milk, but it furnished at least five times as much "folic acid" as the latter. Thus the superior resistance of the whole wheat diet might be tentatively explained by this increased amount of "folic acid." However, since dried potatoes furnished three times as much "folic acid" as did whole wheat, and yet mice reared on a diet in which potatoes replaced whole wheat did poorly, it is doubtful that "folic acid" played an important role in the natural resistance observed in the experiments reported.

The genetic constitution of the animals appeared to play a much greater role in natural resistance than the diet of the mice. The observations described were carried out in W-Swiss mice, animals which were by no means homozygous. By strict inbreeding and selection the investigators have found that resistance to S. enteritidis partakes of many of the attributes of mendelian heredity. They have found that the influence of heredity is such that after the inbred and selected stocks of mice have achieved a reasonable degree of homozygosity, prediction can be made that members of a given strain will live or die when subjected to the test of infection. In contrast to the experience already cited with W-Swiss genotype mice in which the natural resistance was influenced by the nutrition of the host, the same nutritional factors were found ineffective in influencing the natural resistance when tested in genotypes characterized by a long history of brother-sister inbreeding and selected for resistance and susceptibility respectively.

The authors have thus demonstrated that a diet is capable of conditioning natural resistance, but that this depends upon the genetic constitution of the mice employed.

RELATION OF GENETIC AND ENVIRONMENTAL FACTORS TO THE VITAMIN CONTENT OF FRUITS AND VEGETABLES

Garber (*Science* **101**, *288* (*1945*)) has discussed recent advances in plant breeding in relation to human nutrition. It is pointed out that the plant breeder has devoted his

major efforts toward increasing production at lower cost, and although general aspects of quality have not been neglected, breeding specifically for nutritive value is relatively new. This latter objective has become a major activity of the U. S. Southeastern Regional Vegetable Breeding Laboratory and of several experiment station projects cooperating with the U. S. Department of Agriculture.

This journal has already referred to the great differences which may exist in the niacin content of corn varieties (*Nutrition Reviews* **3,** *26* (*1945*)). Values from 8 to 62 micrograms per gram were observed, and it seemed probable that genetic factors were prominently involved in these differences. A nearly classic illustration of the relation of vitamin content to genetic factors was provided by Mangelsdorf and Fraps (*Science* **73,** *241* (*1931*)) in studying the carotene content of dent corn. A quantitative relation was noted between the inheritance of yellow endosperm and carotene content, and the four possible endosperms resulting from crosses of a yellow and white dent corn contained 7.5, 5.0, 2.25, and 0.05 units of carotene per gram, following the gene factorial composition.

Poole, Grimball, and Kanapaux (*J. Agr. Research* **68,** *325* (*1944*)) have shown that hereditary factors are most important in producing strains of cabbage with different ascorbic acid contents, and Wade, Heinze, Kanapaux, and Gaetjens (*J. Agr. Research* (*in press*)) have segregated certain snap bean crosses with a high ascorbic acid content, and indicate that control of a limited number of hereditary factors should lead to a new variety with a distinctly higher ascorbic acid content. Scott and Belkengren (*Food Research* **9,** *371* (*1944*)) emphasize nutritional quality as a goal for breeding programs in the case of peas and corn. They analyzed a number of canning varieties of peas for carotene, vitamin C, thiamine, niacin, and riboflavin. These varied in the extremes from 1.4 to 4.2 micrograms of carotene per gram, 120 to 340 for ascorbic acid, and 1.4 to 4.3 for thiamine. Variations were smaller for niacin and riboflavin. In the case of corn, carotene values ranged from as low as 1 microgram per gram to 7 micrograms.

Harper and Zscheile (*Food Research* **10,** *84* (*1945*)) have illustrated the variations in carotene content which may occur in carrot varieties. A survey of sixteen commercial varieties ranged from 27 to 71 micrograms per gram dry weight. These investigators have utilized accurate spectrophotometric methods for the determination of the extracted carotene, and found that 40 to 50 per cent of the total carotene may be in the form of the less biologically active alpha-derivative. This is in contradistinction to the common belief that a negligible proportion is in this form, and is of importance in utilizing total carotene figures as a measure of provitamin A values. The generally lower carotene values reported by these workers for the 1943 season led to the suggestion that weather or soil conditions may also be of importance in determining vegetable carotene values, at least of carrots.

Adams and Smith (*U.S.D.A. Misc. Pub. no.* **536** (*1944*)) have summarized the reports of various experiment stations with respect to varietal differences of the vitamin content of fruits and vegetables. As an illustration of the gain which may be realized by extensive surveys of fruit varieties, the work of the Arkansas Station on peaches may be cited. Seventeen varieties of peaches ranged from 20 to as high as 500 micrograms of carotene per 100 g. of fresh fruit. Apples, although generally recognized as a poor source of ascorbic acid, could in the case of certain varieties represent an important source of dietary ascorbic acid. A New Zealand report (Kidson, *New Zealand J. Sci. Tech.* **25B,** *134* (*1943*)) cites the Sturmer variety of apple as containing approximately 35 mg. of vitamin C per 100 g., and a variety

has been reported from Geneva, N. Y. (Stotz, *Farm Research* **11,** (*no. 1*) *2* (*1945*)) which is nearly as rich as the orange in this vitamin. Such an "apple a day" has great economic as well as nutritional significance.

Among the small fruits, which may contain relatively large amounts of ascorbic acid, great variations may also be found. Hansen and Waldo (*Food Research* **9,** *453* (*1944*)) report that a series of commercial varieties and seedling selections of strawberries vary widely from 52 to 107 mg. of ascorbic acid per 100 g., from 17 to 38 mg. in the case of red raspberries, and from 12 to 28 mg. for blackberry and dewberry varieties and selections. Some of the selections originated by breeding were found to contain more ascorbic acid than commercially grown varieties, and there is every indication that a further breeding program will lead to high vitamin varieties of small fruits.

In addition to genetic influences on the nutritional qualities of fruits and vegetables, the environmental factor is receiving increased attention. In the work of Hansen and Waldo with small fruits, an apparent correlation of the ascorbic acid content of strawberries with the amount of sunlight previous to harvesting was noted. This effect was studied under more controlled conditions by shading the plants with frames constructed with single or double layered muslin. In other plots only the clusters of berries were covered with muslin bags. The effect of deep shading was to cause a decrease from 80 mg. vitamin C per 100 g. in the unshaded group to 47.6 mg. Lighter shading showed a less marked effect (64.4 mg.) and shading the berries caused an even smaller decrease (70.9 mg.).

In tomatoes, Kaski, Webster, and Kirch (*Food Research* **9,** *386* (*1944*)) have also attributed seasonal fluctuations in ascorbic acid to differences in previous periods of light intensity. A more intensive study by Hamner, Bernstein, and Maynard (*J. Nutrition* **29,** *85* (*1945*)) leaves little doubt that the amount of sunlight previous to harvesting has a marked effect on the ascorbic acid content of the fruit. A possible magnitude of this effect is indicated by the twofold to threefold range in ascorbic acid content of the same variety of tomato grown at different locations in the country. When tomato plants were shaded by cheesecloth (75 per cent decrease in illumination) at first blossom, the fruit contained 15.5 mg. of ascorbic acid per 100 g. as compared with 25.8 mg. in the unshaded controls. Other sets of plants were shaded when the fruit reached the ¼ inch diameter stage, the 1 to 2 inch stage, and when mature green. The mature red fruit from these attained the same low value of those which were shaded for the longer growing period. In reverse, plants grown in the shade, but transferred to sunshine at these periods of growth, developed mature fruit characteristic of those grown for the whole period in sunshine. It is evident then that the effects of sunshine occur just previous to the ripening period.

In so far as ripening in storage is concerned, these investigators found that tomatoes ripening during the first week of storage contained as much ascorbic acid as those freshly harvested, but if they ripened during the second or third week of storage, considerable decreases were observed. As much as 50 per cent of the ascorbic acid may be lost if ripening occurs only in the third week of storage. These workers therefore believe it desirable to harvest tomatoes after a few days of bright sunny weather, and that canning regions with consistently high light intensity would be advantageous.

Realizing the maxim that a processed product can be no better in nutritive value than its raw material, and believing that good nutrition will best result from intelligent selection of foods, an effort to select varieties of fruits and vegetables with high food value represents an important nutritional advance.

DIETARY FAT AND HEPATIC TUMOR FORMATION

The replacement of 5 per cent corn oil in a synthetic diet by hydrogenated coconut oil decreases the incidence of hepatomas in rats resulting from ingested *p*-dimethylaminoazobenzene from about 60 per cent to 8 per cent or less (*Nutrition Reviews* **2,** *338 (1944)*). An attempt has been made to find a plausible explanation for the inhibitory effect of hydrogenated coconut oil in the great chemical differences which exist between this oil and corn oil (Miller, Kline, Rusch, and Baumann, *Cancer Research* **4,** *153, 756 (1944)*). Hydrogenated coconut oil contains no unsaturated fatty acids whereas corn oil is highly unsaturated, linoleic acid constituting approximately 42 per cent of its fatty acid content. Yet the protective effect of hydrogenated coconut oil persisted in the presence of amounts of ethyl linolate sufficient for the growth and well-being of rats on a fat free diet. Secondly, the fatty acids in hydrogenated coconut oil are of relatively short chain length, lauric acid in ester form being present in greatest quantity. But when pure synthetic trilaurin was fed, at a level equivalent to the lauric acid in 5 per cent hydrogenated coconut oil, only slight protection against hepatoma formation was obtained, thus indicating that the effect of hydrogenated coconut oil must be due to factors other than lauric acid glycerides. This is further borne out by the observation that raw coconut oil was less anticarcinogenic than hydrogenated coconut oil. Alpha-tocopherol, which is much more abundant in corn oil than in coconut oil, had no effect when added to the hydrogenated coconut oil, while the removal (by a chromatographic technic) of antioxidants from either oil was also without effect.

Another possibility considered is that the azo dye may be partially destroyed *in vitro* or in the gastrointestinal tract in the presence of hydrogenated coconut oil. However, chemical analyses revealed that the azo dye was stable in the diets *in vitro*. Destruction in the gastrointestinal tract appears improbable since hydrogenated coconut oil is not an autoxidizable oil and the mixture of hydrogenated coconut oil and alpha-tocopherol, which is equally anticarcinogenic, certainly is even more resistant to oxidation. Absorption of the dye does not appear to be altered since analyses again failed to reveal any great difference in the amount of dye remaining in the digestive tract, whether hydrogenated coconut oil or corn oil was fed. A high tumor incidence obtained with a diet free of added fat indicates that dietary fat is not indispensable for the action of the dye.

The mechanism of the anticarcinogenic action of hydrogenated coconut oil thus remains obscure. It is perhaps significant that the rats fed hydrogenated coconut oil were observed to eat more food and gain more weight than rats fed corn oil. Possibly, as a consequence, the rats fed coconut oil would have a higher liver glycogen content. An adequate concentration of glycogen in the liver is well known to protect the liver against toxic agents. Liver glycogen analyses would, therefore, be of interest.

EYE CHANGES IN HUMAN ARIBOFLAVINOSIS

There has been considerable debate concerning the specificity of the changes in the eye which Sydenstricker, Sebrell, Cleckley, and Kruse (*J. Am. Med. Assn.* **114,** *2437 (1940)*) attributed to riboflavin deficiency. This subject was reviewed previously (*Nutrition Reviews* **2,** *120 (1944)*) when the observations of Vail and Ascher (*Am. J. Ophth.* **26,** *1025 (1943)*) were discussed in some detail. These workers pointed out

that any form of inflammation of the conjunctivae may lead to congestion in the meshwork about the cornea and that to regard this reaction as a specific sign of riboflavin deficiency was unjustified. They agreed, however, that such deficiency may sometimes be the primary or secondary cause of changes in the cornea.

Mann (*Am. J. Ophth.* **28,** *243* (*1945*)) calls attention to the great discrepancy in the alleged incidence of ariboflavinosis. Tisdall, McCreary, and Pearce (*Canad. Med. Assn. J.* **49,** *5* (*1943*)) reported ocular signs of ariboflavinosis in 197 of 198 Royal Air Force personnel examined. Gregory (*Brit. Med. J.* **2,** *134* (*1943*)) on the other hand, found only 31 cases in 1059 patients, and Ferguson (*Lancet* **I,** *431* (*1944*)) found 17 instances among 250 outpatients. Scarborough (*Brit. Med. J.* **2,** *601* (*1942*)) found a proportion intermediate between the Canadian and British reports, namely, 70 instances of eye signs among 204 patients.

Dr. Mann remarks that the reports in the literature seem to separate themselves into two groups and to depend on whether the observers were ophthalmologists and therefore conversant with the appearance of the limbus under different conditions and cognizant of the different kinds of corneal vascularization. A distinction should be made between the filling up of preexisting but empty vessels and real proliferation of vessels on the cornea. The latter is a condition of great rarity and is probably of great significance in recognizing ocular signs associated with ariboflavinosis. She criticizes the failure, in the literature, to discriminate between circumcorneal injection and ciliary congestion and between a full limbus and an actual invasion of the cornea by new vessels. The earliest certain sign of riboflavin deficiency, she believes, is a budding out of new capillaries from the limbal loops at their apexes, with extension on to the true cornea. This should be present in both eyes and around the whole corneal circumference.

The case reported by Mann is of interest because it is accepted by a well known ophthalmologist as one of ocular ariboflavinosis and because certain changes not heretofore reported are described. The patient complained of red and sore eyes and even tenderness and pain. The skin at the corners of his mouth was dry and scaly, and there was ulceration of the lower gum. Though the diet had been poor, the tongue was clean and red. Study by the Oxford Nutrition Survey revealed a low blood riboflavin (17 micrograms per 100 ml. plasma) and poor dark adaptation.

Examination of the eyes revealed a distinct new formation of corneal vessels round the whole circumference in both eyes as well as opacities in the cornea, concentrically arranged just beyond the tips of the new vessels and between these and the papillary margin. In the neighborhood of the opacities the vessels passed more deeply. When examined by slit-lamp the corneal lamellae in the opacities appeared to be split. All of these changes are illustrated in color in Mann's article.

The relationship of the lesions to riboflavin deficiency is suggested by the rapid emptying of the vessels under riboflavin treatment. Even four months after treatment was given, however, the new vessels, though completely emptied of blood, were just visible by retroillumination with the slit-lamp and the corneal opacities were still evident although they were narrower and showed a brown pigmentation. Mann regards the picture in this patient as so characteristic that it could not be easily diagnosed as anything else, and certainly not as either rosacea keratitis or interstitial keratitis—conditions with which ocular ariboflavinosis has been confused.

VITAMIN C AND PHYSICAL EFFICIENCY

The great clinical writers on scurvy have described lassitude and a change in complexion as the earliest indications of the approaching onset of this disease in adults. (For illustrative quotations from the works of Lind and of Hess *see Nutrition Reviews* **3,** *44, 45 (1945).*) This raises the question whether there can occur in the body a degree of vitamin C deficiency which is insufficient to lead to the appearance of clinical scurvy, but might cause a decrease in physical fitness.

Crandon, Lund, and Dill (*New Engl. J. Med.* **223,** *353 (1940)*) observed that in a subject who was maintained on a diet containing no vitamin C but believed to be otherwise complete, a feeling of fatigue began in the third month on the deficient diet and became progressively more marked, although no other sign of scurvy appeared until the sixth month of the experiment. Fatigue tests made when scurvy had appeared showed an impairment of strength and endurance as compared with the results of similar tests made after treatment with ascorbic acid.

Johnson, Darling, Sargent, and Robinson (*J. Nutrition* **29,** *155 (1945)*) have extended our knowledge of the effect of a vitamin C free diet on physical fitness in a well planned experiment on a number of young adult male subjects working in a forestry camp in the mountains of New Hampshire. After a preliminary period in which all the volunteers received an excellent normal diet for two weeks, one group was given a vitamin C free diet and placebo capsules; a second group received the deficient diet and capsules with ascorbic acid (75 mg. daily); a third group was given a normal diet and the placebo; and a fourth group received the normal diet and ascorbic acid capsules (75 mg. daily). This regimen was continued for eight weeks, after which the first two groups reverted to normal diet. All subjects received yeast extract daily "to guard against any possibility of vitamin B deficiency."

Care was exercised to destroy all traces of vitamin C in the deficient diet, and to record the food intake and physical activity of each subject. Weekly "pack tests" to assess physical efficiency (fitness for hard work), periodic physical examinations, routine blood and urine analyses, ascorbic acid determinations, and interviews to determine whether the subjects suffered any complaint due to this diet, were carried out during the period of the experiment. The results appear to be completely negative, since none of the subjects on the deficient diet (all except one of whom were engaged in manual work) showed any deterioration in physical vigor, inefficiency in his daily work, or symptoms or signs attributable to lack of vitamin C. Thus, as far as it went, the experiment confirmed the work of Crandon, Lund, and Dill.[8] The ascorbic acid level of the blood serum of the subjects on the deficient diet fell to zero, and these subjects became "unsaturated" with regard to ascorbic acid. However, it has been pointed out by Pijoan and Lozner (*New Engl. J. Med.* **231,** *14 (1944)*) and by Dann and Darby (*Physiol. Rev.* **25,** *326 (1945)*) that neither of these findings is necessarily indicative of any departure from normality.

Johnson and co-workers stress that their observation that eight weeks of complete deprivation of vitamin C has no effect on physical efficiency applies only to groups similar to the one they studied: normal, healthy young men, working in a temperate environment and receiving adequate calories and ample amounts of all other essential nutrients apart from vitamin C. However, there is no reason to believe that physical efficiency would be impaired more rapidly by lack of vitamin C if there was a partial lack of some other dietary essential from the diet, and the findings reported may be expected to apply to many population groups dissimilar to the one they studied.

THE NITROGEN LOSS IN EXUDATES IN SURGICAL CONDITIONS

The focusing of interest on convalescence has revealed a number of interesting problems and important observations. It is now well recognized that a substantial nitrogen deficit occurs following trauma (*Nutrition Reviews* **3,** *86 (1945)*). The loss of protein in the exudate from surface burns has received the attention of several groups of workers (Lucido, *Ann. Surgery* **111,** *640 (1940);* Taylor *et al., Ibid.* **118,** *215 (1943);* Co Tui *et al., Ibid.* **119,** *815 (1944);* Hirshfeld *et al., Surgery* **15,** *755 (1944)*). Just how important from a nutritional standpoint is the nitrogen loss in exudates in various surgical conditions is indicated by the quantitative study of Co Tui and co-workers (*Ann. Surgery* **121,** *223 (1945)*).

As might be expected, many difficulties were encountered in collecting the material from an actively exuding surface. The method finally adopted consisted of the use of slabs of fine cellulose sponge which were rendered nitrogen-free. Such sponges were usually left in contact with the exuding surfaces for from twenty-four to forty-eight hours, at the end of which time they were transferred to dilute sulfuric acid for digestion. Great care was taken to make as accurate collections as possible.

The size of the nitrogen loss was impressive. It ranged from 1.2 to more than 9.57 g. of nitrogen in twenty-four hours. This is equivalent in protein to 7.5 to 59.8 g. If this much protein is lost in cases of burns, avulsion, and local infective conditions such as lung or liver abscesses, the civilian hospital diets are inadequate to support nutrition in such cases. The basic or standard diet recommended by the National Research Council on the basis of Sherman's survey in 1920 contains 70 g. of protein per kilogram of body weight or 11.2 g. of nitrogen. A "high protein" diet consisting of from 120 to 130 g. of protein and supplying 19 to 21 g. nitrogen does not always answer the nutritional problem because pain, loss of appetite, and other upsets may reduce the actual intake. Special measures of alimentation, such as those offered by the use of amino acid mixtures orally and parenterally, may be required.

Other findings in this work were also of interest. There seemed to be a rough parallelism between the percentage of body surface involved in burns and the magnitude of nitrogen loss. By polarimetry the authors were able to calculate the losses in cases of burns per unit of area. The highest figure they observed was 3.28 mg. of nitrogen loss per square centimeter of surface. At this rate an average person with 50 per cent of his 1.8 square meter of surface involved in burns could lose 185 g. of protein in twenty-four hours, an amount which would deplete the blood almost entirely of its principal osmotic component. In addition to the size of the surface involved, other factors play a role. Thus protein loss appeared to depend on whether the wound was second or third degree and on how much tissue had been converted into an eschar which would seal off exudation until it was sloughed off. The observations revealed a sharp distinction between clean and infective operative surfaces for in the former there appeared to be a tendency to seal off exuding points rapidly whereas in infected surfaces the same could not be expected.

The observations reported represent a random study. A more complete investigation involving a continuous record not only of the nitrogen loss in the exudates, but also in the urine and feces is indicated. The study is of value in that it should call to the attention of the practicing physician the need for an awareness not only for the vitamin needs but also for the caloric and nitrogen needs of the patient, particularly in conditions which involve large losses of proteinous fluids.

NOTES

City-Wide Nutrition Clinic

Announcement is made of the establishment of a city-wide nutrition clinic at the Lower East Side Health and Teaching Center of New York City. It is planned that the Public Health Research Institute and the Nutrition Division of the Department of Health will cooperate with the nutrition clinic to correlate the dietary and chemical studies with the medical findings.

Nitrogen Balance in Burns

This subject has been reviewed (*Nutrition Reviews* **3,** *2 (1945)*), but a new publication by Croft and Peters (*Nature* **155,** *175 (1945)*) is of such interest that it deserves immediate attention. In this preliminary report the authors are concerned with the urinary loss of nitrogen following severe burns, which occurs in rats, as well as in human patients. The negative nitrogen balance occurring in burned rats maintained on a diet containing 14 per cent of protein could be largely eliminated by increasing the dietary protein level to 22 per cent.

In attempting to account for the great loss of nitrogen from the body the authors followed the working hypothesis that possibly only one amino acid was especially needed for the repair of the burned area, and that this was made available by the breakdown of other healthy body tissue in large quantities; the amino acids not especially needed for repair would then be deaminized and give rise to the excessive excretion of urea. Experiments with rats, based on this hypothesis, have shown that the excessive nitrogen loss after burns is prevented as effectively by including 1 per cent of dl-methionine in the diet as by raising the protein level from 14 to 22 per cent. This beneficial result was not obtained with some other amino acids.

It is pointed out that the reason for the increased need for methionine is still unknown, as is the way in which it is utilized. However, this new finding makes "unnecessary the view that the nitrogen losses in burns are due to toxemia, as they can be explained upon the basis of deficiency." The excessive loss of nitrogen by burned human patients has been checked by the ingestion of very large amounts of protein (Taylor *et al.*, *Ann. Surgery* **118,** *215 (1943)*) but in many cases the patient's appetite is too poor to allow ingestion of an adequate amount. It is suggested that such patients might be promptly aided by treatment with methionine, but the necessary clinical trials have not been reported.

Nutrition in Industry

A lucid account of the organization and accomplishments of the various federal agencies which have to do with the industrial feeding program was presented by Goodhart to the Twenty-second Annual Conference of the Milbank Memorial Fund (*Milbank Memorial Fund Quart.* **23,** *78 (1945)*). The magnitude of this program is indicated by the statement that "by January 1, 1945, in-plant feeding should be available to a total of 12 million." Among the list of "tasks requiring attention," Goodhart has included "studies on the relation of nutritional status to toxic reaction in industry."

The meager and unsatisfactory state of our knowledge of this latter problem is indicated by the paper which Crutchfield presented to the same group (*Milbank Memorial Fund Quart.* **23,** *97 (1945)*). This presentation is replete with such generalizations as "it is well known that general undernourishment or a deficiency of some vital element of nutrition increases fatigue, and it certainly increases susceptibility to infection. I do not feel that it is a long jump from this demonstrated fact to the assumption that such nutritional deficiencies increase susceptibility to toxic exposures. . . . Although

no reference has been found in the literature on the prophylactic use of a low-fat diet in workers exposed to nickel, such a procedure seems to be indicated and is one which might be used very effectively." The experience with vitamin C in lead poisoning and the results of Holman and Donnelly (*J. Exp. Med.* **76,** *511 (1942)*) which demonstrate that the kidney of the dog is protected from experimental mercuric chloride poisoning by a previously induced hypoproteinemia, should serve to indicate the caution with which one must regard such untested generalizations (*Nutrition Reviews* **1,** *66, 298 (1943);* **2,** *178, 261 (1944)*).

Conservation of Nutritive Value of Foods

A series of conferences in the National Cooperative Project on the Conservation of Nutritive Value of Foods was concluded by a meeting in New York City in February, by representatives of the agricultural experiment stations of the Northeastern Region. Previous conferences had been held by experiment station workers on this project from the Southern, North Central, and Western Regions.

This National Cooperative Project was inaugurated for the purpose of coordinating experiment station research dealing with factors affecting the nutritive value of foods, particularly those foods of importance in the national nutrition program. The project is under the general administration of the Office of Experiment Stations of the United States Department of Agriculture. Work in each of the four geographic regions is coordinated by one of the state experiment station directors of that region.

Annual regional conferences have been held for the purpose of the evaluation of the results on various commodities, the consideration of methodology, and for the planning of future work. The research findings on this project have been published in the form of confidential progress notes, seventy-nine of these reports having been issued during 1943 and 1944. A plan is under way to make the final results generally available through their publication under the headings of separate commodities, as bulletins of the state agricultural experiment stations.

Pernicious Anemia and Vitamin Bc

The common factors of relationship between vitamin Bc, "folic acid," and xanthopterin and the macrocytic hypochromic anemia in chicks have been reviewed previously (*Nutrition Reviews* **2,** *131 (1944)*), although the specific nature of the substance or substances whose deficiency is responsible for the chick anemia has not been elucidated. The possibility that vitamin Bc might be related to the elusive extrinsic factor in human pernicious anemia is logical because of the physical association in natural sources of the vitamin B-complex and Castle's extrinsic factor.

Results of therapy with concentrates of vitamin Bc made from yeast and liver, given by mouth to patients with pernicious anemia in relapse, have been uniformly negative according to a report by Sharp and Vonder Heide (*Proc. Central Soc. Clin. Res.* **17,** *41 (1944)*). Five of their 6 patients had been followed for a period of years and their responses to oral administration of potent liver preparations were standardized. The other patient was observed for the first time in the study under consideration, hence no control period was available for comparison.

Levels of Bc in the urine of 3 of the patients and suitable nonanemic controls indicated that the factor was utilized, stored, or perhaps eliminated in the feces. During four weeks of the study none of the patients showed any hematologic changes indicative of a true remission while vitamin Bc was given by mouth in doses ranging from 440 to 1500 micrograms daily. Although larger doses or preliminary incubation with normal gastric juice might have been effective, under the condition of the test "vitamin Bc did not induce erythropoietic maturation in relapsed pernicious anemia." It is apparent that further tests are required for final evaluation of vitamin Bc.

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The Nutrition Foundation was organized by manufacturers of the food industry to carry out a program of public service.

The purposes of the Foundation are:

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2. The development of an educational program to facilitate the widest possible usage of what is known in the science of nutrition.

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